THE CONTROL OF CEMENT GLAND DEVELOPMENT IN THE CRAYFISH, CAMBARUS [1]

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The previous work concerned with elucidating possible hormonal mechanisms in the control of secondary sexual characteristics in the Crustacea has been recently reviewed by Brown (1952). That such work has to date been unsuccessful in providing indisputable evidence for an influence of endocrine secretions on these characteristics may be explained in part by reference to technical obstacles in the path of a direct application of standard endocrinological techniques to decapods. The position of the gonads in this group, together with problems imposed by the brittle, calcified exoskeleton, have rendered gonadectomy so difficult that it has not yet been successfully attempted. Takewaki and Nakamura (1944) were able to castrate an isopod, *Armadillidium,* but found no apparent influence on the development of the oöstegites in females.

The second basic problem encountered in attempting to study hormonal influences on the development of secondary sexual characteristics lies in the nature of the characters which have been considered. Those secondary characters which appear cyclically in relation to the breeding season would appear, *a priori,* to be those most readily influenced by fluctuations in hormonal balance. However, those which have been studied in this connection to date have the disadvantage of appearing as discontinuous modifications in form at the time of molt. Thus, their occurrence tends to be of an all-or-none character which renders studies of developmental rates difficult.

The cement glands of the Astacura may be considered secondary sexual characteristics on the basis of their sexually dimorphic character and the intimate relation between their discharge and the process of egg-laying. They are usually assigned the function of producing the cement which attaches the eggs to the pleopod setae of the female. Reviews of earlier literature concerning the function of these glands are provided by Broekhuysen (1936) and Yonge (1937).

Andrews (1904) described the process of egg-laying and glaire secretion in *Cambarus affinis* and stated that the cement glands in this form are chiefly found ventrally beneath the abdominal sterna and in the abdominal appendages. Farkas (1927) conducted a detailed histological study of the glands in *Astacus* and stated that the cement glands are essentially similar to the other tegumental glands described in his work. Two types of cells can be distinguished in the glands: relatively large duct cells and smaller secretory cells. The duct cells produce an intra-

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cellular system of ducts which supplies the secretory cells and provides a pathway for the discharge of their mucin-like secretion.

These glands are particularly suitable for studies of possible hormonal influences on their development since their maturation occurs as a continuous process without the necessary intervention of molt. Yonge (1937) suggested the possibility of humoral control of their development but did not attempt to support this possibility. Lloyd and Yonge (1940) considered a correlation noted between the mature condition of the ovary and the cement glands in *Crangon* to be evidence for the existence of a hormone elaborated by the ovary and responsible for cement gland development. No attempt has been encountered to study possible hormonal influences on the cement glands using classical endocrinological techniques.

An important reproductive function of the crustacean sinus gland was first demonstrated for the shrimp, *Leander*, by Panouse (1943, 1944, 1946) and subsequently confirmed for *Cambarus* and *Uca* by Brown and Jones (1947, 1948). In these forms, sinus gland removal results in an increased rate of ovarian maturation. The work of Stephens (1952) provides evidence for a role of materials elaborated by the cerebral ganglia and circumesophageal connectives in the sexual cycle of *Cambarus*. Consideration of these results directed attention in the following work to the sinus gland and the central nervous system as possible sources of tropins concerned with cement gland development.

Material and Methods

The experiments to be described were performed with groups of *Cambarus rusticus* obtained from Ohio and with groups of *Cambarus virilis* collected in the vicinity of Chicago, Illinois. Reference is also made to scattered observations of other species of *Cambarus* collected locally. The protocol of each of the several experiments performed will be described subsequently.

The operative procedures were: (1) removal of both eyestalks, and (2) implantation of central nervous organs in the ventral abdominal haemocoel. The eyestalks were removed at their bases with a scalpel, thus removing the sinus glands and associated structures. The eye stubs were cauterized immediately to avoid excessive blood loss. In the case of animals receiving implants of central nervous tissues, the cerebral ganglia and circumesophageal connectives were removed from donor animals and implanted at once in a medium of van Harreveld's solution with a tuberculin syringe bearing a 16-gauge hypodermic needle. Animals were not fed in the course of any experiments.

The distribution of the cement glands will be described for female *Cambarus rusticus*. No differences in distribution were observed in other species of *Cambarus* employed. The glands underlie the second through the sixth abdominal sterna and extend laterally into the anterior region of the associated pleura. They also occur in the protopodites, endopodites and exopodites of the last five pairs of abdominal appendages. The pores through which the cement glands discharge their secretion open independently of the setae of these appendages.

In order to assess the results of experimental treatment on the development of the cement glands, a series of arbitrary stages based on their gross appearance at various points in their development has been used. Although the gross appearance as described for each stage is much the same in all of the cement glands in a given animal, it is convenient to confine attention to the ventral surface of the uropods. The stages have been defined as follows:

Stage 1: The distinguishing characteristic of this stage is the appearance of milky-white, translucent, circular or subcircular areas, approximately 0.1 to 0.2 mm. in diameter, marking the position of the future cement gland pores. There may be 30 to 45 such areas on each ramus of a uropod depending to some extent on the size of the animal. An examination of sectioned material indicates that these areas are correlated with the appearance of the distal duct cells of the cement glands. A considerable number of ducts is associated with each presumptive pore, each duct being only about five micra in diameter.

Stage 2: The white areas described above persist and subcircular, transparent areas appear within their confines ranging from 0.05 to 0.1 mm. in diameter (Fig. 1). These transparent areas are the cement gland pores which penetrate through the chitinous layers of the integument to the non-chitinous epicuticle (Fig. 4).

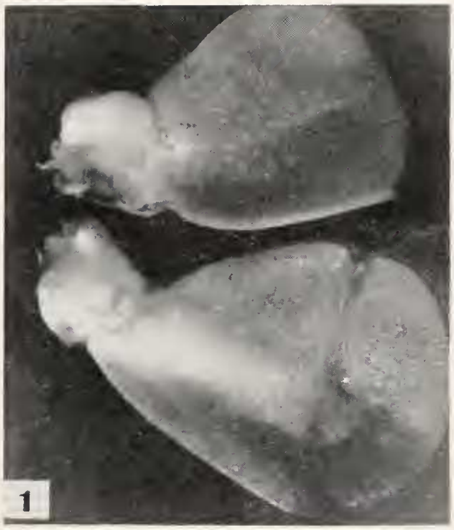
Stage 3: At this stage the secretory cells have appeared in close association with the inner duct cells and are just beginning secretory activity. The glands can be seen as translucent, white, lobate clusters surrounding the cement gland pores in an irregular fashion. Each lobe represents a discrete gland, many glands providing independent ducts to one pore. Figure 3 is a photograph of a histological section of cement glands at this stage.

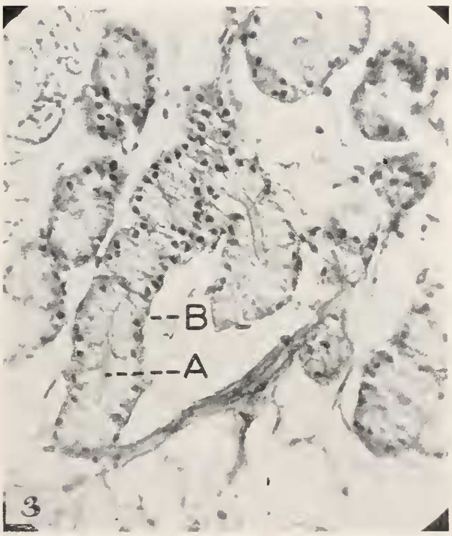
Stage 4: The previously described elements are still clearly visible and in addition the cement glands have become striking, opaque, cream-white as a result of engorgement with dense mucoid secretion. Figure 2 illustrates the gross appearance of cement glands which are approaching this stage. However, the cement glands eventually become so heavily charged with secretion and so distended that it is difficult to make out their lobate character. Stage 4 is the terminal or mature stage of the cement glands.

Intermediate stages of cement gland development can be defined quite accurately. Thus an animal with patent stage 1 areas plus about half the cement gland pores would be classified as stage 1.5. Such interpolations allow staging to quarter units throughout the scale. The accuracy of such determinations has been ascertained by re-reading groups of animals within an interval so brief that no significant change could have occurred. Using a group of 10 animals, the average reading checks within 0.05 unit.

One source of error was encountered in using this system. When experimental conditions are such that no cement gland development is occurring, the translucent areas surrounding or portending the pores may fade. Since they normally limn the pores, superficial examination may fail to reveal all the pores present which would produce a low reading. It was also ascertained that these areas disappear just following molt but normally reappear within 48 hours after molt.

The fading which is observed in animals which are not in immediate post-molt stages can be considered retrogressive development or it can be interpreted as a concomitant of developmental arrest. The latter alternative has been chosen in the analysis of data in this paper. Thus decrements measured in comparing stages of the same animals taken at different times are reported but are simply treated as zero increments in the calculation of averages. The alternative procedure would





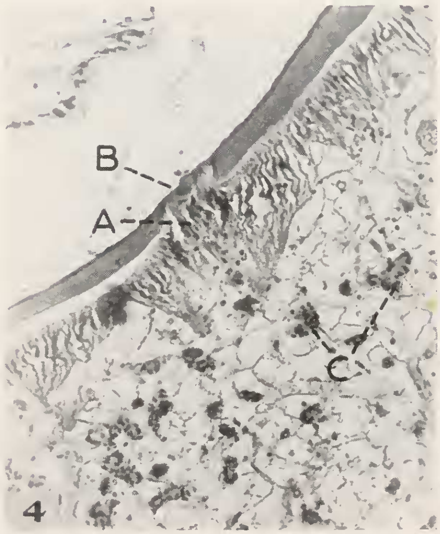


FIGURE 1. Gross appearance of the uropod of a female *Cambarus rusticus* with the cement glands at stage 2.0 (× 8).

FIGURE 2. Gross appearance of the uropod of a female *Cambarus rusticus* with the cement glands at stage 3.5 (× 8).

FIGURE 3. Histological section through uropod showing cement glands at stage 3.0. The ducts (A) can be seen lying within the masses of gland cells (B) (× 220).

FIGURE 4. Histological section through uropod showing the cement gland ducts (A) and a cement gland pore (B). Gland cells (C) which have appeared in association with the inner duct cells but have not yet begun secretion may be seen in this section (× 120).

not materially influence the conclusions suggested by the data. Animals which had molted less than two days previous to the time of a reading were not considered. Finally, it should be stressed that development as evaluated by means of the scale described above is not known to proceed at a uniform rate.

Experiments and Results

A. Influence of destalking and central nervous system implants

In the course of these experiments, it became apparent that the mature *Cambarus rusticus* females employed were responding to experimental treatment in a quite different fashion from the small *Cambarus virilis* females. It will be argued later that these *C. virilis* were immature. In any event, it is simplest to discuss the experiments employing these different species separately.

1. Cambarus rusticus

A preliminary experiment was performed over the period from July 4 to July 13, 1951. Three groups of females ranging in carapace length from 2.5 to 3.9 cm. were employed.

Group A consisted of 40 animals of average carapace length 3.26 cm., destalked on July 4. Group B consisted of 40 animals of average carapace length 3.26 cm., similarly destalked on this date and receiving one implant of nervous tissue from female *C. rusticus* donors on July 8. Group C was comprised of 15 untreated animals whose average carapace length was 3.13 cm. and served as a control group. All animals were maintained in separate compartments in running water at an average temperature of 22.6° C.

The staging system for evaluating the condition of the cement glands was not yet developed at the time these animals were isolated and consequently no values are available for the initial condition of their cement glands. Readings of each surviving animal in each group were taken on July 13, nine days after destalking and five days after implanting the groups so treated. These observations are arranged in tabular form in Table I.

A trend toward more advanced cement gland development with increasing size is apparent in all groups. However, there is considerable variation within a given size range. In an attempt to compensate for this variation, the average cement gland stage has been calculated for a series of overlapping size ranges and is presented graphically in Figure 5. This graph shows that the average development of the cement glands in those animals which were destalked and received nervous tissue implants is quite consistently more advanced than the condition manifested by the controls and those animals which were destalked but received no implants.

An additional control group was selected from the stock animals on July 13 and the cement glands staged. Although the variation between control groups was considerable, both control groups were clearly less advanced in cement gland development than the implanted animals. These results are suggestive of a possible tropic influence of the nervous system on cement gland development. In order to test this possibility further, the following experiments were performed.

Experiment 1. Two groups of female *Cambarus rusticus* ranging in carapace length from 1.7 to 2.8 cm. were considered in this experiment. Group A consisted of 14 animals, destalked on August 22, whose average carapace length was 1.92 cm. with an average cement gland stage of 0.77. Group B consisted of 14 animals which were destalked on August 22 and received one implant of nervous tissue

TABLE I

Initial experiment with Cambarus rusticus. Condition of the cement glands of each animal in each group on July 13, 1951

Carapace length (cm.)	Destalked	Cement gland stage destalked, implanted	Control
2.5	1.5		
2.6			1.0
2.7		1.5	2.75
2.8	0.5 1.0		1.5 1.0
2.9	1.0 3.0 1.25	1.5 2.0	1.75 1.5
3.0		2.25 1.25 3.0 3.0	3.0
3.1	1.25 1.5	2.0 2.0 1.5	1.25
3.2	2.0	3.0 2.0 1.5	3.0
3.3	3.25 3.0 3.5 1.5 1.5	3.0	3.0
3.4	3.0 2.0 3.0	3.25 3.5	
3.5	2.0	3.5	2.0
3.6	1.5 3.0 3.0 3.5	3.0	2.75 2.25
3.7	2.0		
3.8	3.25	1.75	3.0

from female donors on August 26. The average carapace length of the animals in this group was 2.19 cm. and their average cement gland stage was 1.14. These animals were maintained in separate compartments in running water at an average temperature of 22.5° C. The state of the cement glands was assessed on August

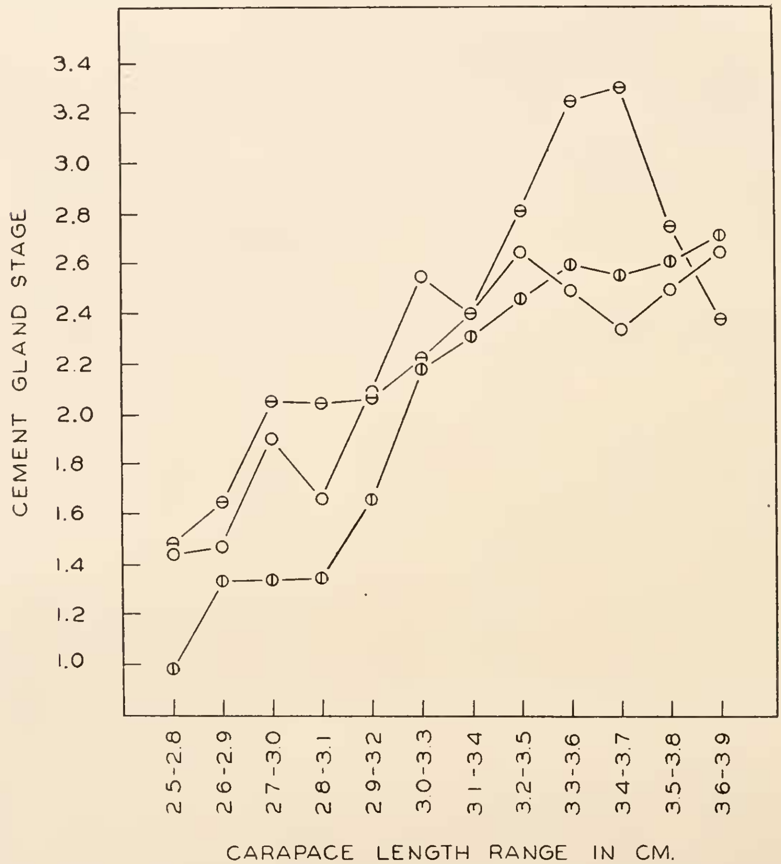


FIGURE 5. Graph showing the average cement gland stage on July 13, 1951 for the size ranges indicated of animals in groups A, B, and C of the initial experiment with *Cambarus rusticus*. Group A (destalked) ⦶. Group B (destalked, implanted) ⊖. Group C (untreated controls) ○.

26 and again on August 31. The increments for the animals in each group are tabulated in Table II. The average increment for each group was:

Group A (destalked, 9 surviving) 0.53
Group B (destalked and implanted, 9 surviving) 0.45

It should be pointed out at this time that group A in this experiment is not comparable to any other group considered because of its low initial cement gland condition.

Table II

Increment in cement gland stage measured over the period indicated in experiments with C. rusticus and C. virilis

Cambarus rusticus

Experiment 1		Experiment 2		
Group A (destalked)	Group B (implanted)	Group A (destalked)	Group B (implanted)	Controls (9/6 to 9/14)
1.25	0.25	0.25	1.25	0.0
0.25	1.25	0.0	1.0	0.25
1.0	0.25	0.5	0.25	0.0
0.25	0.25	0.75	0.0	0.0
0.5	0.0	0.75	1.0	0.0
0.75	0.75	0.0	1.5	0.0
0.0	1.0	0.25	1.25	0.25
0.75	0.25	0.5	1.0	−0.25
0.0	0.0	0.25	0.75	−0.25
		0.0	0.0	−0.25
				−0.25
Av. = 0.53 ± 0.15	Av. = 0.45 ± 0.15	Av. = 0.325 ± 0.092	Av. = 0.80 ± 0.17	Av. = 0.05 ± 0.032
$\sigma = 0.44$	$\sigma = 0.445$	$\sigma = 0.29$	$\sigma = 0.54$	$\sigma = 0.101$

Cambarus virilis

Experiment 1		
Group A (destalked)	Group B (implanted)	Group C (control)
0.0	0.0	0.0
0.0	0.0	0.0
−0.25	−0.25	0.0
−0.25	−0.25	0.0
−0.25	0.25	0.0
		0.0
Av. = 0.0 ± .0	Av. = 0.05 ± 0.06	0.0
		0.25
$\sigma = .0$	$\sigma = 0.12$	0.25
		0.25
		0.25
		0.25
		0.25
		0.25
		Av. = 0.125 ± 0.036
		$\sigma = 0.13$

Experiment 2. Two groups of *Cambarus rusticus* ranging in carapace length from 1.7 to 3.2 cm. were considered in this experiment. Group A consisted of 11 animals destalked on September 20. The average carapace length of animals in this group was 1.89 cm. and their average cement gland stage was 1.66. Group B consisted of 20 females which were destalked on September 20 and received one implant of nervous tissue from female donors on September 22. The average

carapace length of this group was 2.10 cm. and their average cement gland stage was 1.63.

These animals were maintained in separate compartments in running water at an average temperature of 19.6° C. The condition of the cement glands was assessed on September 22 and again on September 28. The increments for the surviving animals in each group are tabulated in Table II. The average increment for each group was:

Group A (destalked, 10 surviving) 0.325
Group B (destalked and implanted, 10 surviving) 0.80

A control group of 14 untreated animals was maintained over the period from September 6 to September 14 to provide information concerning the normal rate of cement gland development under the laboratory conditions described. These animals were maintained in running water in separate compartments at an average temperature of 21.2° C. The condition of the cement glands was assessed on the dates mentioned above and the increments for the surviving 11 animals are tabulated in Table II. The average increment for this group was 0.05.

Before concluding the presentation of the results of these experiments, one important fact should be mentioned. The increments reported in state of cement gland development in destalked animals and in animals receiving implants after destalking are not the initial portion of a continuing increase in developmental state but rather an induced spurt of development which does not persist until the glands attain maturity. Mortality in these experiments was high and consequently observations are scattered, but no instance has been observed where induced development involved a total increment of more than 1.5 stage units. In general, no increment, or only a very small one, is measured after an initial ten-day to two-week period of response. Animals have been maintained as long as 45 days after destalking and show no substantial advance over their cement gland condition 10 days after this operation.

2. *Cambarus virilis*

Experiment 1. Three groups of *Cambarus virilis* females collected from a small artificial lake in the vicinity of Chicago, Illinois were employed in this experiment. Their carapace lengths ranged from 2.0 to 3.0 cm.

Group A consisted of 20 animals which were destalked on July 29. The average carapace length of the animals in this group was 2.48 cm. and their average cement gland stage was 1.44.

Group B consisted of 20 animals which were destalked on July 29 and received one implant of nervous tissue from female *C. virilis* donors on July 31. The average carapace length of the animals in this group was 2.54 cm. and their average cement gland stage was 1.46.

Group C was comprised of 20 untreated animals whose average carapace length was 2.54 cm. and whose average cement gland stage was 1.44. This group served as normal control for groups A and B.

All animals were maintained in separate compartments in running water at an average temperature of 23.0° C. The developmental condition of the cement glands was assessed on July 29 and again on August 8, 10 days after eyestalk removal and 8 days after implanting the groups so treated. The increments for the animals in each group over this period are tabulated in Table II. The average increment for each group was:

Group A (destalked, 5 surviving)	0.00
Group B (destalked and implanted, 5 surviving)	0.05
Group C (normal controls, 14 surviving)	0.125

Experiment 2. This experiment was performed with a total of 90 locally collected female *C. virilis* ranging in carapace length from 1.9 to 2.8 cm. The animals uniformly showed cement glands at stage 2.0. They were divided into five groups as follows:

1. Destalked on November 1.
2. Destalked on November 1 and received one implant of nervous tissue from female donors on November 3.
3. Destalked on November 1 and received one implant of nervous tissue from male donors on November 3.
4. Destalked on November 1 and received an implant of muscle tissue on November 3.
5. Untreated controls.

All animals were maintained in separate compartments in running water at an average temperature of 17.0° C. The condition of the cement glands was assessed on November 1 and again on November 13. No fewer than 11 animals in each group survived this period. In no case did any animal manifest any advance in cement gland condition, all animals remaining at stage 2.0. Subsequent implantation of nervous tissue from mature *Cambarus propinquus* female donors was similarly without effect on the cement gland development of the host *C. virilis*.

The following observations seem to indicate that the animals employed in this experiment were immature. The oöcytes in these animals were small, pale, translucent white with an average maximum observed diameter (23 animals, carapace length 2.0 to 2.7 cm.) of 0.237 mm. This is to be contrasted with the presence of olive-green oöcytes of diameters ranging from 1.5 to 2.0 mm. in normal mature *C. virilis* at this time of year. In addition, the gonopods of the males of similar size and collected at the same time were uniformly of form II (the sexually inactive or juvenile form) in contrast to the form I (sexually active) gonopods manifested by mature males at this season.

Similarly the oöcytes of the *C. virilis* females employed in the first experiment with this species were small and the gonopods of comparable males were of form II. However, this is less definite evidence of their immaturity since mature females may normally have small, white oöcytes in July. However, mature males are usually in form I by the end of July so we may tentatively argue that these animals were immature. The fact that the cement glands of these animals were in stages less than 2.0 might be taken to indicate that they were hatched in the same spring. However, in the absence of observations of the cement glands of yearling, immature crayfishes, this does not necessarily follow.

B. Additional observations and experiments

Two experiments were conducted to determine if the length of the daily light period to which the animals were exposed influenced the rate of cement gland de-

TABLE III

Cement gland stages of animals subjected to 20-hour light and constant darkness, assessed on the dates indicated

Carapace length	Cement gland stage					
	20-hour light			Constant darkness		
	7/17	7/24	7/29	7/17	7/24	7/29
2.1	1.0	1.0	1.0	0.75	1.0	0.75
2.2	0.75	0.75	1.25	0.75 1.5	1.0 1.25	1.5
2.3	0.75 1.0	1.5	1.5			
2.4	1.0 1.0 1.0	1.5 1.25 1.0	1.5 1.5 1.0	1.0 1.0	1.0 1.0 1.25	1.25
2.5	1.0	1.25		1.0 1.0	1.5 1.5	1.0 1.25
2.6	0.5 1.5 1.0 1.5	1.25 1.25 1.25 1.5	1.5 1.5 1.75 1.75	1.5 1.0 1.5 1.0 1.5	1.5 1.25 1.0 1.25	1.5 1.5 1.25 1.5
2.7	1.0 1.5 1.5 2.0 1.25 1.25 1.0 1.25 1.5	1.0 3.0 1.0 2.0 1.25 1.25 2.0 1.5 1.25	1.25 2.5 2.0 1.5 1.75	1.5 1.5 1.5 1.25 1.25	1.5 1.0 1.75 1.5 1.25	2.0 1.5 1.5 1.5 1.75 1.75
2.8	1.5 1.5 2.0 1.25	2.5 1.75 1.75 1.75	1.5 1.5 1.75 1.5 1.5	1.25 1.0 2.0 1.5 1.0 1.5	1.5 3.0 1.75 2.0 1.25 1.75	1.25 1.5 1.5 1.0 1.5 3.0
2.9	1.5 1.5 1.0	1.5 1.0 2.0	1.75 1.25 2.5 2.0	1.5 1.5 2.0	1.5 1.5 2.0	1.5 1.75 2.0
Av.	1.23	1.49	1.60	1.30	1.45	1.52

velopment. Again there is a sharp dichotomy in the response of the mature *C. rusticus* and that of the immature *C. virilis*.

In the first experiment two groups of female *C. rusticus* were maintained in running water, one in constant darkness and the other in 20-hour daily illumination. Group A, in 20-hour light, consisted of 28 females of average carapace length 2.61 cm. and an initial average cement gland stage of 1.23. Light was provided by two 25-watt incandescent lamps suspended from a wooden frame approximately 10 inches above water level, producing an illumination of from 8 to 50 foot-candles at the surface of the water. The lights were controlled by an automatic time clock and the tank shielded with black cloth to prevent the access of undesired illumination. Group B consisted of 26 animals of average carapace length 2.63 cm. and average initial cement gland stage 1.30 and was maintained in constant darkness. The animals in these two groups were not isolated. The average water temperature over the experimental period for group A was 23.0° C. while that for group B was 22.3° C.

The cement glands of each animal in each group were assessed on July 17, July 24 and July 29. These observations are tabulated in Table III. The increments may be expressed as units per day since the two periods are not the same. The averages are:

Twenty-hour light	
July 17 to July 24	0.037
July 24 to July 29	0.017
Constant darkness	
July 17 to July 24	0.021
July 24 to July 29	0.010

The cement glands of the animals exposed to 20-hour light seem to have developed more rapidly than those of the animals maintained in darkness for both periods. In both cases, development for the first period was more rapid than for the second. Observations were discontinued on July 29 because of an increase of mortality. Since the animals were not isolated, readings of only a portion of the original sample would be of dubious value.

A second experiment of similar design was initiated on November 7 with small *C. virilis* (average carapace length 2.21 cm.) whose cement glands were uniformly at stage 2.0. It was found that alteration of daily light ration did not suffice to induce development beyond this point in any of the animals.

The extent of cement gland development was positively correlated with the size of the animals in all groups examined with the exception of groups where all of the animals were at maturity and the *Cambarus virilis* referred to above whose cement glands were uniformly at stage 2.0. Examination of groups of *C. virilis, C. propinquus, C. immunis, C. rusticus, C. diogenes* and *C. blandingii acutus* indicates that this positive correlation is widespread among crayfishes. The type of evidence obtained is illustrated by Figure 5 where cement gland stage is plotted against carapace length. *C. rusticus* provided the largest size range over which this effect could be observed. As early as July 13, large females (carapace length of 4.5 cm.) were found with typical mature cement glands while on the same date an animal of carapace length of 1.5 cm. might show no trace of these structures.

A correlation between ovarian development and cement gland development was observed in *C. rusticus*. Ovarian condition was estimated by measuring the largest observed oöcytes with an ocular micrometer and a binocular dissecting microscope and recording this size as well as the color of the oöcytes. Such measurements were

TABLE IV

Maximum observed oöcyte diameter in millimeters listed according to cement gland stage of the animals

Stage 1.0 −1.75	Stage 2.0 −2.75	Stage 3.0 −3.75	Stage 4.0
0.5	0.6	0.65	1.0
0.5	0.65	0.8	1.0
0.9	0.9	0.8	1.2
0.45	1.2	1.1	0.9
0.5	0.7	0.7	1.0
0.5	0.85	0.8	
0.7	0.7	0.9	
0.45	0.8	0.9	
0.4	0.55	0.8	
0.45	0.6	0.7	
0.55	0.8	1.0	
0.6	0.9	1.0	
0.6	0.6	0.9	
0.5	0.4	0.9	
0.45	0.9	1.0	
0.5	1.1	0.8	
0.6	0.6	0.85	
0.5	0.6	0.8	
0.45	0.65	0.9	
0.4	0.9	1.0	
0.6	0.6	0.4	
0.6	0.6	1.3	
0.7	0.7	0.5	
0.7	0.8	0.9	
0.7	0.7	0.8	
0.6	0.8	0.8	
0.7	0.7	1.0	
0.7	0.8	1.1	
0.7	0.7	0.7	
0.9	0.8	1.0	
0.6	0.7	0.3	
0.45	0.75		
0.6	0.6		
0.5	0.65		
	0.4		
Av. 0.575±0.019 σ=.114	0.720±0.028 σ=.165	0.842±0.036 σ=.202	1.02±0.049 σ=.11

made for one hundred and five *C. rusticus* females at all stages of cement gland development from stage 1.0 to stage 4.0. These measurements are grouped according to cement gland development stage and tabulated in Table IV. The average values for these groups indicate that ovarian and cement gland development proceed in a roughly parallel fashion, though there is a considerable range of variation within each group. Similar data for other species of *Cambarus* indicate that this correlation is probably general in occurrence.

Discussion

It may be of value to provide a brief outline of events in the reproductive cycle of female crayfishes to form a basis for interpreting the results presented above. Reproductive cycles in *Cambarus* in the North Temperate Zone may be divided into two types on the basis of the time of egg-laying. Eggs are always hatched in the spring but may be laid in the fall and carried over the winter (*Cambarus immunis*, Tack, 1941), or they may be laid in the spring (*Cambarus propinquus*, van Deventer, 1937). In either case, the females present in a population in mid-summer may be divided into three groups:

1. Those animals which were mature and bore a clutch in the previous breeding season.
2. Those animals which failed to attain a minimal size necessary for reproductive activity in their first year, yearling immature females.
3. Those animals which were hatched in the preceding spring. This group can be logically subdivided into animals which will and those which will not attain the critical breeding size.

The cement glands of mature females revert to stage 0 (no gross indication of their presence) after the process of egg-laying. It is not known whether they begin to re-form immediately or there is a time lag before re-initiation of development. The cement glands of the yearling immature females attained stage 2.0 by the time of entrance into hibernaculae the preceding year. It is not known whether they continue their development from this point at the onset of spring or revert to stage 0 to re-initiate development of the cement glands. The third group of animals hatched in the preceding spring attains a stage of 2.0 or 4.0 by the following fall depending on their rate of growth and their success or failure to attain a minimal size for reproductive activity.

This cycle suggests that the correlation between size of the animals and extent of cement gland development observed during this study is not necessarily the result of a differential rate of development alone but may possibly be explained in part in terms of the three groups of animals in a population, outlined above.

Rates of cement gland development observed in unoperated animals may be listed to provide a basis for discussing variations induced by experimental operative treatment. These rates are expressed as average increments in cement gland stage per day.

Control group, *C. rusticus*	0.012
Control group, *C. virilis*	0.005
Twenty-hour light, *C. rusticus*	0.037
	0.017
Constant darkness, *C. rusticus*	0.021
	0.010

These rates may be compared with the *per diem* rates for destalked *C. rusticus* females and females receiving central nervous implants subsequent to destalking.

Experiment 1:	destalked	0.106
	destalked and implanted	0.090
Experiment 2:	destalked	0.054
	destalked and implanted	0.133

It is apparent that all of these rates clearly exceed any of the rates of cement gland development listed for unoperated animals. Thus the conclusion that destalking induces an increased rate of development of the cement glands seems justified. That this is not the result of the loss of the eyes in their capacity as photoreceptors is shown by the rate manifested by the unoperated animals in constant darkness. We may then conclude that some factor originating in the eyestalk, probably in the sinus gland, is serving to inhibit cement gland development in these animals.

The case for a central nervous tropic influence on the development of the cement glands rests on the initial exploratory experiment performed with *Cambarus rusticus* and on the results of the second experiment providing the data listed above. It was pointed out in describing experiment 1 with this species that the group of animals which was destalked was not comparable to any of the other groups considered because of the low initial stage of the cement glands. That this may have influenced the results is indicated by the fact that those animals with cement glands initially at stages less than 1.0 exhibited greater increments in this experiment than those with more advanced glands. However, although a role of central nervous neurosecretory elements in the control of cement gland development may be suggested, further experimental verification is necessary.

In no case did destalked *Cambarus virilis* females, whether receiving implants or not, exhibit significant cement gland development. The single increment measured in the implanted group of the first experiment (Table II) is within the limit of error inherent in the technique of estimation. It follows that removal of the sinus glands in these animals produced a cessation of development or failed to initiate development as contrasted with the increment in rate produced with similar treatment of *Cambarus rusticus*. Either the differences between the responses of *Cambarus rusticus* and *Cambarus virilis* to operative treatment are the result of a species difference in the controlling mechanism of cement gland development, or they are related to the fact that all of the *C. rusticus* were mature and all of the *C. virilis* were immature. In view of the close systematic relationship of the two forms, the latter explanation seems more probable.

These facts taken together suggest a controlling mechanism for cement gland development which closely parallels that postulated by Stephens (1952) for the control of ovarian development in *Cambarus virilis*. Thus the sinus gland would serve in the role of inhibitor (as shown by the increment in developmental rate in destalked, mature animals) and also elaborate a substance necessary either directly or indirectly for the development of the cement glands (as shown by the cessation of development in destalked, immature animals). The central nervous system in mature animals would serve either to store the sinus gland tropin or to convert it to an active form and store it, the rate of its release being under the control of the sinus gland inhibitor. A system with these characteristics would explain all of the reported observations but requires further experimental support.

Callan (1940) and Knowles and Callan (1940) demonstrated the failure of secondary sexual characteristics to develop in females of *Leander* following parasitic or x-ray castration. They suggested the possibility of an ovarian hormone controlling these characters but did not rule out the possibility of a general somatic effect of such treatment. On the other hand, Takewaki and Nakamura (1944) demonstrated that surgical removal of the ovary in the isopod, *Armadillidium*, did not influence subsequent oöstegite development.

Although a correlation is reported here between the state of ovarian and cement gland development, discrepancies in this correlation actually provide evidence against the gonadal hormone theory. The presence of early stages of cement gland development concomitant with an advanced ovarian condition might be explained in terms of a failure of tissue competence by a proponent of the gonadal hormone theory. However, in Table IV, two cases are presented in the third column which show a clear discrepancy in the other direction. These animals had cement glands in stages 3.0 and 3.5 and had white oöcytes of maximum observed diameter of 0.4 and 0.3 mm., respectively. The occurrence of such animals with advanced cement glands and small, immature oöcytes would appear to argue strongly against a direct dependence of the cement glands on the ovary. It may also be pointed out that Brown and Jones (1947) report a linear increase in dry-weight of the ovaries of destalked *Cambarus immunis* over a 53-day period which contrasts sharply with the short burst of development of the cement glands followed by relative quiescence produced by similar treatment. Thus it can be tentatively concluded that the control of the cement glands, at least, is not mediated by an ovarian tropin.

Summary

1. The morphology and sequence of events in the course of normal development of the cement glands of female crayfish of the genus *Cambarus* are described and a technique for the study of the developmental rate of these glands is outlined.

2. In mature female crayfish, removal of the eyestalks produces an acceleration in the rate of development of the cement glands. Experimental evidence suggests that a further increment in rate may result when cerebral ganglia and circumesophageal connectives from mature females of the same species are implanted in the ventral haemocoel of destalked, mature animals.

3. Both destalking, and destalking with subsequent implanting of nervous tissue from immature female donors, lead to a cessation of cement gland development or fail to initiate development in immature animals.

4. The cement glands of mature females subjected to 20 hours of illumination per day appear to develop more rapidly than those of comparable animals maintained in constant darkness. However, modification of day-length is not sufficient to initiate cement gland development in quiescent immature females.

5. A correlation between developmental state of the cement glands and degree of oöcyte development is presented.

6. Characteristics of a possible humoral control of the cement glands are discussed as well as the contribution of this information to the more general subject of humoral control of secondary sexual characteristics in the decapods.

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